Diverse roles for ubiquitin-dependent proteolysis in transcriptional activation

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A growing literature points to a fundamental role for the ubiquitin–proteasome degradation system (UPS) in transcription. Four recent publications add significant insight to our understanding of the connections between these processes. Each provides evidence that some aspect of the UPS can stimulate the activity of transcriptional activators. UPS might promote transcription by several mechanisms, and in some cases, even the final step of the UPS — proteolysis — might enhance activator function.

Transcription is an immensely complex process. The initiation step alone involves the assembly of dozens of factors to form a pre-initiation complex (PIC). Assembly of this juggernaut is nucleated by the recognition of a DNA sequence within chromatin by a transcriptional activator protein. Subsequently the activator recruits various coactivators and the RNA polymerase holoenzyme. Finally, once this network of interacting polypeptides has been assembled, polymerase must be released from the promoter to execute elongation. Factors left behind at the promoter may then catalyse another round of transcription or the PIC may disassemble and be rebuilt from scratch.

Adding even more complexity to this process are recent results implicating the ubiquitin–proteasome degradation pathway (UPS; Box 1) in transcription (for a detailed review see ref. 1). In the simplest example of a link between these processes, degradation can limit an activator's abundance and therefore its activity, as is true of p53 (ref. 2), β-catenin[3], glucocorticoid receptor (GR)[4] and Rpn4 (ref. 5). However, numerous observations have hinted at a more intimate connection between the UPS and transcriptional activation. For example, activators are often unstable proteins whose transcriptional potencies are correlated with rates of turnover[6]. In addition, the sequences within activators that specify proteolysis largely overlap with the transcriptional activation domains[7], and components of the transcription machinery recruited by activation domains[8] can promote the destruction of activators[9,10]. There is even evidence that the UPS controls transcription by mechanisms that do not involve protein turnover. For example, ubiquitination of VP-16 seems to stimulate its capacity for transcriptional activation[11], and a subcomplex of the proteasome might promote transcription by using a chaperonin-like activity[12] to remodel chromatin or transcriptional complexes[13].

Now, four recent papers lend even more excitement to this rapidly expanding niche of ubiquitin biology. The first report suggests that ubiquitin-mediated proteolysis stimulates transcription promoted by the oestrogen receptor (ER)[14]. Two other manuscripts characterize a role for the UPS in the function of the Myc proto-oncoprotein[15,16]. Finally, the most recent effort proposes that the proteasome performs a non-proteolytic task at the *CDC20* promoter[17]. The remainder of this review will discuss these recent contributions and develop a model that attempts to synthesize present thinking on the mechanistic links between the UPS and transcription. Although we consider both proteolytic and non-proteolytic functions of the UPS in transcription, we focus our attention on potential roles of proteolysis because the non-proteolytic functions of ubiquitin in transcription have been explored in detail elsewhere[1].

Box 1 The ubiquitin–proteasome degradation system (UPS). Ubiquitin-mediated proteolysis entails the covalent attachment of ubiquitin to a substrate (for review see ref. 33). This ubiquitin generally serves as a signal for the subsequent degradation of the substrate protein by the 26S proteasome, but it can have other functions. Ubiquitin is attached to a substrate polypeptide by means of a series of enzymatic reactions involving E1, E2 and E3 enzymes. After activation by the E1, ubiquitin is then transferred to an E2 enzyme. The E2 then binds to an E3-ubiquitin ligase to form a complex that attaches ubiquitin to a lysine residue on the substrate. Additional rounds of transfer to the lysine-48 residue of ubiquitin result in a chain of ubiquitins that targets the substrate for proteolysis by the 26S proteasome. The proteasome consists of the 20S core that contains the peptidase activities plus the 19S regulatory cap. The cap is divided further into lid and base[34]. The base comprises a ring of six ATPases (Rpt1–6) that are thought to unfold and translocate substrate into the lumen of the 20S particle. The lid–base interface and the lid harbour, respectively, a putative ubiquitin chain receptor (Rpn10) and an isopeptidase (Rpn11) that cleaves ubiquitin chains from substrates[35,36].

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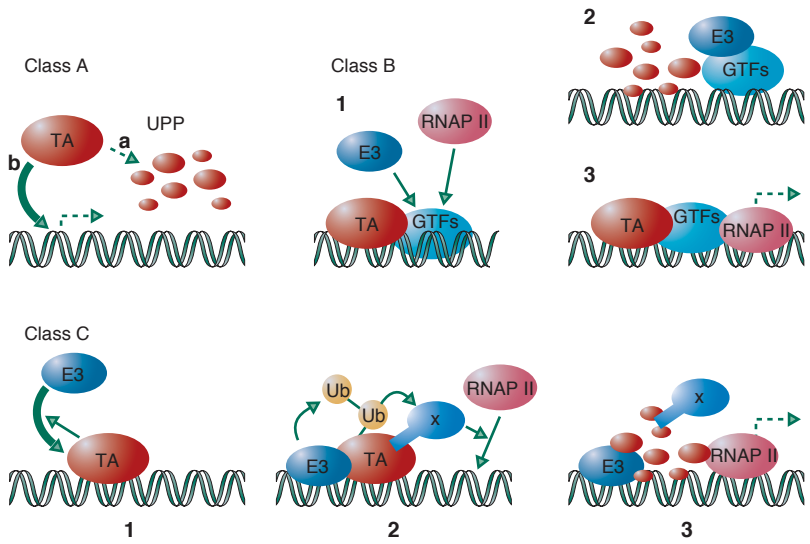

Figure 1 Class A, B and C transcription factors. Class A: transcriptional activator (TA) is degraded by the ubiquitin-proteasome system (UPS). Activator output is determined by the relative rates of degradation (a) and transcriptional activation (b). Class B: degradation of activator requires binding to DNA and possibly a subsequent step in the assembly of the pre-initiation complex. The rate of activator degradation determines the window of time available to stimulate transcription. In the example shown, DNA-bound activator recruits general transcription factors (GTFs), which in turn recruit both E3 ligase and RNA polymerase (RNAP) (1). If E3 is recruited first, activator degradation short-circuits transcription (2). If polymerase is recruited first, transcription ensues (3). Class C: a specialized form of class B in which activator degradation actually stimulates transcription. In the example shown, activator recruits an E3 ligase 'coactivator' (1) that ubiquitinates the activator, enabling the association of GTF(X) to complete PIC assembly (2). Subsequent degradation of the activator triggers the release of elongating polymerase (3).

Proteolysis stimulates transcription

Previous findings established that blocking proteolysis with proteasome inhibitors diminishes the ability of ER and the androgen receptor (AR) to activate transcription[18,19]. To investigate the basis of this effect for ER, Reid *et al.*[14] combined a FRAP (fluorescence recovery after photobleaching) assay[20] with chromatin immunoprecipitation (ChIP) analyses. In a remarkable series of ChIP experiments, liganded and unliganded ER were shown to orchestrate the cyclical recruitment of ubiquitin ligases and proteasome ATPases (but not 19S lid or 20S subunits) to an ER-responsive promoter. Interestingly, blocking transcription with Pol II inhibitors stabilizes ER, whereas proteasome inhibitors inhibit ER-responsive transcription and immobilize ER within the nuclear matrix. Reid *et al.*[14] propose that transcription-dependent ER ubiquitination recruits a proteasome ATPase subcomplex that then transfers ubiquitinated ER to the nuclear matrix, where it is degraded by the 26S proteasome. This is an intriguing hypothesis, but it is unclear how the accumulation of ER in the nuclear matrix in the presence of proteasome inhibitor would affect transcription at an ER-responsive promoter. In contrast with findings for ER and AR, proteasome inhibition stimulates the transcription of glucocorticoid receptor (GR) targets, even though GR also accumulates at the nuclear matrix[4]. It will be interesting to unravel why the functions of these nuclear hormone receptors are modulated by the UPS in different ways.

The ubiquitin pathway activates transcription

Closely following Reid *et al.*, two other papers[15,16] report on the ubiquitination, turnover and activity of the proto-oncoprotein, Myc. Both papers provide strong complementary evidence that Skp2, a subunit of an E3 ubiquitin ligase complex, is required for ubiquitination and degradation of Myc. The most compelling observations are the following: first, endogenous Myc and Skp2 interact at the time that Myc is degraded; second, Myc is stabilized in cells depleted of Skp2 by siRNA; and third, post-translational activation of Skp2 induces Myc turnover. Remarkably, even as Skp2 promotes Myc ubiquitination and turnover, it induces the expression of Myc-responsive genes and ectopic entry into S phase in wild-type but not $myc^{-/-}$ cells. Conversely, Myc can no longer activate its target genes in $skp2^{-/-}$ cells. Buttressing these functional data are ChIP analyses showing that Skp2, an unidentified ubiquitinated protein, and components of the proteasome 20S core, 19S lid and 19S base bind the promoter of the Myc target gene *CYCLIN D2*. These tantalizing results argue that the Myc and Skp2 proto-oncoproteins act together at promoters to induce the expression of Myc target genes, which in turn push the cell cycle into S phase. Because neither paper directly addressed the effect of Myc proteolysis, a complete picture of the interplay between Myc ubiquitination, proteolysis and transcriptional activation awaits future studies.

A non-proteolytic role in transcription?

An even more recent paper that links the UPS with transcription comes from Morris *et al.*[17], who investigated the transcription of *CDC20* in yeast . These authors report that the cyclin-dependent kinase Cdk1 associates cyclically with the *CDC20* promoter during the cell cycle, and this association is negatively correlated with *CDC20* transcription. The Cdk1-associated factor Cks1 recruits proteasome components to the *CDC20* promoter, where they are proposed to remove Cdk1 to facilitate transcription. Similarly to the study by von der Lehr *et al.*[15], but in contrast with studies by Reid *et al.* and Gonzalez *et al.*[13,14], Morris *et al.*[17] detect 19S lid and base components as well as 20S core components at the *CDC20*, *GAL1* and *PUR5* promoters. Mutations in a 19S base subunit cause a modest (about 40%) decrease in *CDC20* transcript, whereas mutations in 20S subunits have no effect. From these data, Morris *et al.*[17] argue that the intact proteasome acts in a proteolysis-independent manner to displace Cdk1 from the *CDC20* promoter, thus activating transcription.

This paper, together with work on Gal4-responsive promoters[13] and ER-responsive promoters[14], indicates that the proteasome or a subcomplex consisting of the ATPase subunits of the 19S base might control transcription by an unknown mechanism that does not involve proteolysis. Although this is an exciting hypothesis, a few cautionary points are worth considering. First, the UPS can selectively degrade single polypeptide chains of multisubunit complexes[21]. As an integral part of this process, the proteasome must extract a ubiquitinated substrate from its partners, unfold it and thread it into the chamber of the 20S proteasome to be degraded. In fact, even when 20S protease activity is decreased, the 26S proteasome can segregate active cyclin/Cdk1 from ubiquitinated Cdk inhibitor Sic1 (ref. 22) and

Table 1 Classifications of selected unstable transcriptional activators

Class	Requirements for turnover and effect of blocking ubiquitination or turnover	Transcriptional activator	Notes	References
A	Degradation of class A activator occurs in the cytoplasm or nucleus but does not require binding to DNA or PIC formation. Inhibition of ubiquitination or turnover leads to increased activity	p53	DNA damage-dependent phosphorylation stabilizes and activates	2
		β-Catenin	Wnt signal-mediated loss of phosphorylation stabilizes and activates	3
		Rpn4	Negative feedback loop: activates proteasome genes, and degraded by proteasome. Proteasome inhibition activates	5
		Glucocorticoid receptor (GR)	Proteasome inhibition immobilizes in nuclear matrix and activates	4
		c-Jun	MAPK signalling stabilizes and activates	37
		Hif1α	Loss of E3 (VHL) increases target gene expression	38
B	Degradation of class B activator requires binding to DNA and possibly a step in PIC assembly. Inhibition of proteasome predicted to enhance activity, but inhibition of ubiquitination may lead to decreased activity	VP16*	Fusion to ubiquitin rescues transcription defect of E3 mutant	11
		Myc*	Depletion of E3 function reduces transcriptional activation, whereas overexpression of E3 activates	15,16
		Gcn4	Mediator component, Srb10, targets Gcn4 for UPS	9
		Ste12	Srb10 also promotes Ste12 degradation	10
C†	Degradation of class C activator requires binding to DNA. In addition, degradation is required for a step in the transciption cycle. Inhibition of ubiquitination or turnover is predicted to decrease activity	Androgen receptor (AR)	Proteasome inhibition immobilizes; represses activation	19
		Liganded oestrogen receptor	Proteasome inhibition suppresses target gene expression and ER mobility	14

*Because the effect of inhibiting turnover was not tested directly, it is not possible to distinguish unambiguously between class B and class C for these activators.
†The existence of a class C activator has not yet been unambiguously proven.

Cdk1 from ubiquitinated cyclin B[23]. Similarly, in a proteasomal mutant, unfolding of the key (unknown) target that is processed by the proteasome at the *CDC20* (ref. 17) and *GAL1* (ref. 13) promoters might be uncoupled from its degradation. However, this need not mean that such uncoupling normally occurs in an unperturbed cell. Second, although the absence of proteasome 20S and lid subunits at promoters has been interpreted as evidence for a novel proteasome ATPase subcomplex[13,14], it might instead reflect technical limitations of ChIP assays[24]. Consistent with this is the observation of both 20S and lid subunits at the *CDC20* and Myc-responsive promoters[15,17]. Last, in interpreting the effects of 20S proteasome mutations it should be noted that although there is considerable evidence that the degradation of Sic1 is important for entry into S phase in budding yeast, none of the proteasome mutations except *nin1-1* (ref. 25) exhibit a defect in S-phase entry. The spectrum of proteins and processes affected in a proteasome mutant might simply reflect the relative abilities of different substrates to compete for a dwindling proteasome activity. We suggest that until more is known, the provocative hypothesis that a proteasome subcomplex controls transcription by a novel 'remodelling' mechanism should be evaluated cautiously.

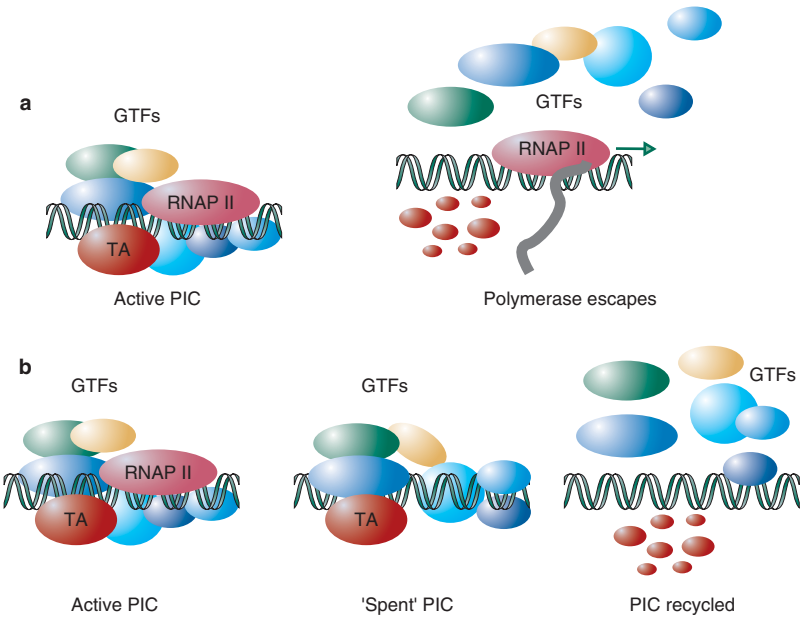

Figure 2 Different mechanisms by which proteasome-mediated degradation might stimulate transcription. (**a**) Ubiquitinated activator is degraded, which destabilizes the pre-initiation complex (PIC) to allow the release of active polymerase. (**b**) Alternatively, some other process (such as RNAPII phosphorylation) instigates initiation, leaving behind a spent (inactive) PIC. Degradation of the activator clears the promoter, enabling the assembly of a new, functional PIC.

Mechanisms for regulating transcription by proteolysis

A conundrum emerges from studies on the functional consequence of ubiquitin-dependent proteolysis of activators. In many cases, proteolysis inhibits activator function, but in some cases proteolysis unexpectedly seems to promote activator function. Why should there be this difference? In the remainder of this article we propose a systematic classification of activators that helps to rationalize how the UPS can exert negative and positive effects on transcription. A key theme is that the timing of activator ubiquitination and degradation with respect to particular events in transcription determines the functional consequence of proteolysis. We suggest that most activators can be segregated into three classes that we designate classes A, B and C (Table 1): class A activators are ubiquitinated and degraded regardless of whether they bind DNA, whereas activators from classes B and C are targeted only once they have bound DNA. The activity of both class A and B activators is increased upon inhibition of their turnover, owing to increased concentration of the activator. In contrast, the degradation of class C activators is required to complete a step in the transcription process; obstruction of turnover therefore inhibits transcription. We now describe these classes in more detail.

Class A activators: degradation as a restraint against activity

At one end of the spectrum, consider an activator whose degradation is completely uncoupled from transcription (Fig. 1). For example, the factor might be degraded as it floats about in the nucleus or cytoplasm. This degradation might be constitutive or switched on and off by signals. In either case, turnover would depress the activator's concentration (which would enable concentrations of the activator to respond briskly to abrupt changes in synthesis or turnover) and would therefore be inhibitory. β-Catenin could be an example of this regulatory mode. Blockade of β-catenin turnover by Wnt signalling leads to the rapid induction of target genes[3].

Class B activators: degradation as an egg timer

Activators of the B class are those that are degraded only upon binding DNA. Ubiquitination and degradation of class B activators might furthermore depend on the successful completion of a step in the pathway of PIC assembly (Fig. 1) and could thereby serve as a timer that limits how long the factor remains bound at a promoter. A similar model for class B activators has previously been proposed[9,26]. A potential implication of having such a timer is that it would enable a 'macromolecular proofreading' mechanism responsive to the rate and/or fidelity of PIC assembly: if activator B rapidly nucleates a PIC, it might trigger multiple rounds of transcript synthesis before it is degraded. Assembly into a productive complex might even shield it from degradation. However, if it lingers too long at the promoter before successfully recruiting polymerase, or if it nucleates the assembly of an unproductive dead-end PIC intermediate, activator B might be degraded before it can promote a transcript. An appealing aspect of this regulatory mode is that the degradation machinery selectively targets a subpopulation of DNA-bound activators that are most relevant for transcriptional output. One example of a class B activator might be Gcn4. Gcn4 recruits the Srb–mediator complex to promoters[27]. The Srb10 protein kinase subunit of this complex phosphorylates Gcn4, which marks Gcn4 for ubiquitination and proteolysis[9]. The Srb10 protein kinase subunit of this complex phosphoylates Gcn4, which marks Gcn4 for ubiquitination and proteolysis[9]. At least one step in this process is likely to occur on the DNA, as GCN4 turnover requires DNA binding (Y.C. & R.J.D., unpublished observations). VP16 and Myc also have properties of a class B factor, with the added complexity that their ubiquitination might stimulate transcription-promoting activity in addition to promoting degradation by the proteasome (see below)[11,15,16].

An interesting consequence of the class B (and class C) lifestyle is that DNA-binding sites for the activator would function as 'coenzymes' that promote degradation. Thus, turnover rate would be dictated by the steady-state molar ratio of an activator to its DNA-binding sites. For abundant activators that have few binding sites, turnover might seem sluggish because the stable bulk population would mask the turnover of a small but functionally relevant, promoter-bound pool.

Class C activators: degradation as a stimulatory switch

Class C comprises activators whose degradation stimulates transcription by enabling the execution of a critical step in the transcription cycle (Fig. 1). How might this work? As has been noted previously[28], the PIC is held together by numerous interactions. It might be necessary to disrupt this assemblage to enable RNA polymerase to break free from the promoter and transcribe downstream sequences. This could occur by one of several mechanisms, including destruction of the activator[29] (Fig. 2). Another possibility is that the components of a spent PIC might have to be cleared from the promoter and reassembled after each transcript is initiated, in much the same way that re-firing a single-shot rifle

requires ejection of the spent casing and the insertion of a new bullet (Fig. 2). If this were true, why is the proteasome not required for the transcription of all genes? First, it could be that promoter architecture dictates how difficult it is for Pol II to escape and whether or not the PIC needs to be rebuilt anew for each initiation event. Second, promoters whose output is insensitive to proteasome inhibition might have intrinsic properties that obviate the need for active mechanisms, or they might recruit other factors that act redundantly with the proteasome[28].

According to our depiction, firing off a round of transcription would inevitably require a fresh molecule of class C activator for each transcript produced. This mechanism, though it seems profligate, recapitulates the control of the initiation of DNA replication: a new pre-replication complex is assembled at an origin each time it is fired. Although admittedly a similar 'once and only once' imperative does not exist for transcriptional regulation, an appealing aspect of the 'single-shot' mechanism is that it enforces a tight linkage between the generation of an active transcription factor and the mRNA transcript that it promotes — a sensible strategy for controlling activators that are governed by dynamic signalling pathways. It is important to note that this type of mechanism has not yet been unambiguously described, but its existence is hinted at by the observation that proteasome inhibitors block the cycling of ER at target promoters[14]. Myc[15,16] and VP16 (ref. 11) might prove to be members of this class once the impact of proteolysis on their activity has been explicitly tested.

Embellishments, exceptions, and concluding remarks

Because proteins typically must be ubiquitinated before they are degraded by the proteasome, an opportunity for additional regulatory flexibility is introduced. For example, ubiquitination of a transcription factor might generate a short-lived hyperactive intermediate that is quenched by proteolysis, as has been proposed for VP-16 and Myc[11,15,16]. Alternatively, it is plausible that in some cases ubiquitination and degradation both act positively, with ubiquitination promoting an upstream step in the assembly of the PIC and degradation promoting a subsequent step such as the release of active Pol II. These regulatory strategies might require a mechanism to suppress the processive nature of ubiquitination or to retard the recognition of multi-ubiquitinated activator by the proteasome. Otherwise, the 'window of opportunity' during which the ubiquitinated factor can operate might be too brief to enable transcriptional activation.

The segregation of factors into classes A, B and C does not mean that these hypothetical regulatory modes operate in a mutually exclusive fashion. For example, an activator that is ubiquitinated by a particular ubiquitin ligase only after its binding to DNA and assembly into a PIC (class B or C) might be ubiquitinated by a different ligase as it floats about freely in the nucleoplasm (like class A). The relative contributions of both ubiquitination pathways (and, hence, of the two regulatory modes) might vary as a function of cell type or signalling processes (Table 1; refs 9, 14). Alternatively, one subcellular pool of the activator might be stable, whereas a second pool dynamically turns over[30]. These considerations complicate experimental analyses of the relationship between activator turnover and function, because looking at the turnover of a bulk population of endogenous molecules (or even worse, overproduced proteins) might yield misleading results.

As is inevitable in biology, some activators such as Met4 clearly do not conform to the groupings demarcated here[31]. In yeast cells grown in poor medium, Met4 is ubiquitinated and degraded (and thereby inhibited) in response to excess methionine. In rich medium, however, Met4 is ubiquitinated but not degraded, and the resulting ubiquitinated Met4 activates a distinct subset of genes. Despite the existence of exceptions such as Met4, we believe that some form of classification promotes systematic thinking about how the UPS controls the activity of activators.

What will come next? Interest in the interface between transcription and the UPS is heating up, but this area nevertheless remains largely unexplored. Many key questions remain. How many activators and promoters throughout the genome are regulated by the UPS? How are ubiquitination, sumoylation, acetylation and methylation of lysine residues integrated to control activator function[32]? And how does the regulation of activators by the UPS actually work? To explore the mechanistic links fully, it will be important to combine microarray studies, quantitative modelling, and reconstitution of ubiquitination, degradation and transcription in the same test tube — a daunting but feasible task. □

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